REMARKS

Claim amendments

Claims 1, 10 and 11 have been amended to more clearly indicate that the capsule comprises a porous membrane formed by a polyelectrolyte complex which encapsulates cells which express cytochrome P450 "as a cell membrane-bound protein", wherein the porous membrane of the capsule is permeable to prodrug molecules and the cells are retained within the capsule. As noted in the MPEP:

> By disclosing in a patent application a device that inherently performs a function or has a property, operates according to a theory or has an advantage, a patent application necessarily discloses that function, theory or advantage, even though it says nothing explicit or concerning it. The application may later be amended to recite the function, theory or advantage without introducing prohibited new matter. *In re Reynolds*, 443 F.2d 384, 170 USPQ94 (CCPA 1971); *In re Smythe*, 480 F.2d 1376, 178 USPQ 279 (CCPA 1973). MPEP, 8th edition, section 2163.07(a), page 2100-177.

It is known in the art that cytochrome P450 is a cell membrane-bound protein. For example, Wei *et al.* teach that cytochrome P450 is "an integral membrane protein" (Wei *et al.*, page 971, column 2).

Provisional Rejection of Claims 1-4 and 6-22 under the judicially created doctrine of obviousness-type double patenting

Claims 1-4 and 6-22 are rejected under the judicially created doctrine of obviousness-type double patenting "as being un-patentable over claims 1-24 of US 6,540,995" (Office Action, page 3).

As the Examiner notes, Applicants will address the provisional rejection upon an indication of allowable subject matter in the present application.

Rejection of Claims 1-4, 6-9, 15-19, 21 and 22 under 35 U.S.C. §103(a)

Claims 1-4, 6-9, 15-19, 21 and 22 are rejected under 35 U.S.C. §103(a) as being unpatentable over Tai *et al.* and Merten *et al.* in view of Wei *et al.* "for the same reasons of record as set forth in the office action mailed 11/18/02" (Office Action, page 3). The Examiner

finds Applicants' position that the claimed invention is unobvious in view of the cited art unpersuasive "because the scope of the invention as claimed is not limited to membrane-bound cytochrome P450 but to the retention of cytochrome P450 expressed by cells inside the capsule" (Office Action, page 4).

As pointed out above, the claims have been amended to more clearly indicate that the capsule comprises a porous membrane formed by a polyelectrolyte complex which encapsulates cells which express cytochrome P450 "***as a cell membrane-bound protein***", wherein the porous membrane of the capsule is permeable to prodrug molecules and the cells are retained within the capsule.

The Examiner further states that Merten *et al.* "clearly teaches an encapsulated bioreactor wherein the product produced by the encapsulated cells is retained within the capsule while allowing growth media in, which is comparable to the invention as claimed" and that Tai *et al.* teach that "appropriate semi-permeability is required to allow easy diffusion of secreted gene product without compromising the immunoisolating properties of the membrane" and "control of membrane permeability by adjusting sodium alignate and poly-l-lysine concentration" (Office Action, page 4). The Examiner states that Merten *et al.* and Tai *et al.* "clearly suggested the manipulation of membrane permeability that not only promotes survival of encapsulated cells but control the diffusion of cellular and extra-cellular products" (Office Action, page 4). The Examiner states that "it is not improper hindsight reasoning but Merton and Tai who clearly suggested the manipulation of membrane permeability that not only promotes survival of encapsulated cells but control the diffusion of cellular and extra-capsular products" (Office Action, page 5). The Examiner concludes that:

> it would have been obvious to one of ordinary skill in the art at the time of filing, to modify the teaching of Tai and Merten who teaches the encapsulation of genetically engineered cells with selected membrane permeability, with the teaching of Wei who teaches genetically engineered cells that produces p450 which activates an inert prodrug. One would have been motivated to encapsulate the cytochrome p450 producing cells in order to retain the P450 gene product within the capsule (Marten) or to avoid immune rejection (Tai). One would have a reasonable expectation of success because prior art clearly teaches that manipulation of pore size of capsule membrane is well within the reach of one of ordinary skill in the art (see Merten, page 128, col. 2 para. 3). Thus, invention as

claimed is prima facie obvious in view of combined teaching of cited art of record (Office Action, pages 5-6).

Applicants respectfully disagree. Tai *et al.* and Merten *et al.* clearly teach encapsulation of cells which express ***nontoxic*** products. Tai *et al.* encapsulated fibroblasts which express human growth hormone in order to "provide ***sustained delivery*** of the desired gene product" (Tai *et al.*, abstract, emphasis added). Merten *et al.* encapsulated hybridomas which express IgG that is retained in order to cultivate "***mammalian cells at high densities***" (Merten *et al.* page 127, column 2, emphasis added).

However, cells which express cytochrome p450 convert prodrugs such as ifosfamide and cyclophosphamide (CPA) into ***cytotoxic*** metabolites, and the cytochrome p450-expressing cells become sensitive to the cytotoxic effects of the metabolites. For example, Wei *et al.* teach that "[s]table transfection of rat C6 glioma cells with the P450 2B1 gene rendered the cultured tumor cells sensitive to CPA" (Wei *et al.*, abstract).

One of skill in the art would expect that encapsulated cells, which express cytochrome p450 and which would therefore be densely packaged within the capsules, would not provide for cultivation of the cells at high density or sustained delivery of the converted prodrug, because once the prodrug enters the capsule and is converted to a cytotoxic drug the encapsulated cells would be killed. Thus, one of skill in the art would not be motivated to encapsulate the cytochrome P450 expressing cells of Wei *et al.* and deliver them to a tumor in order to kill the tumor cells because one of skill in the art would reasonably expect that conversion of the prodrug to its cytotoxic metabolites by the cells within the capsules would ultimately kill the encapsulated cells. Particularly since one of skill in the art would reasonably expect that the concentration of the cytotoxic drug present within the capsule would be significantly higher than the concentration of the cytotoxic drug that would be present in the surrounding environment of the non-encapsulated P450-expressing tumor cells described in the Wei *et al.* reference, even though the capsule is permeable to the prodrug. As pointed above, Wei *et al.* show even when cells transfected with the P450 gene and the converted prodrug were ***not*** densely packed into a capsule, such cells were rendered "sensitive to CPA" (Wei *et al.*, abstract).

Clearly, based on the combined teaching of Tai *et al.*, Merten *et al.* and Wei *et al*, one of skill in the art would not be motivated to encapsulate cells which express cytochrome P450 as a cell-membrane protein wherein the capsules have a porous membrane that is permeable to prodrug molecules and the cells are retained within the capsule.

The claimed invention is directed to a capsule comprising a porous membrane formed by a polyelectrolyte complex which encapsulates cells which express cytochrome P450 "as a cell membrane-bound protein", wherein the porous membrane of the capsule is permeable to prodrug molecules and the cells are retained within the capsule. The cytochrome P450 protein is an enzyme which converts a prodrug such as ifosfamide or cyclophosphamide (CPA) into a cytotoxic drug. The pore sizes of the capsule membrane allow prodrug molecules to pass into the capsules where the prodrug molecules are subsequently converted by cytochrome P450 into an activated cytotoxic drug. "Cytotoxic" means that the drug efficiently kills cells. For this reason, prodrugs such as CPA and ifosfamide are used for the treatment of cancer (*e.g.*, specification, page 6, last paragraph). However, these cytotoxic drugs do not distinguish between "good" and "bad" cells, and thus, kills tumor cells and normal cells. Accordingly, the skilled practitioner would have expected that encapsulated cells which are densely packaged within the capsule, would express a high amount of the cytochrome P450 enzyme, and thus, would be killed by the cytotoxic drug produced in high concentrations within the capsules. Indeed, as disclosed in the specification as filed, a drug induced suicide effect was observed in the capsulated (specification, page 24, 1st paragraph). Thus, a skilled practitioner would have expected that a prolonged release of cytotoxic compounds from the capsule for the purpose of ablating tumor cells or treating a tumor could not be achieved because the encapsulated cells which express cytochrome P450 would be killed. However, as disclosed in the specification and as demonstrated specifically in the Examples of the subject application, a continuous conversion of the prodrug into the cytotoxic drug, and thus, long term delivery of the cytotoxic drug by encapsulated cytochrome P450 expressing cells was observed which indicates that the cells survive despite a higher concentration of the cytotoxic drug within the capsule.

This finding would not be expected in view of the cited prior art. Merten *et al.* tested and optimized a polyanion polycation encapsulation process "for the encapsulation and subsequent cultivation of mammalian cells, in particular hybridoma cells" (Merten *et al.*, page

122, column 1). Using a hybridoma cell that produces IgG, Merten *et al.* demonstrate that "the encapsulation process using sodium cellulose sulphate as polyanion and PDMDACC as polycation, is a suitable tool for the ***cultivation of mammalian cells at high densities***" (Merten *et al.* page 127, column 2, emphasis added).

Tai *et al.* propose an alternative strategy for somatic gene therapy (*i.e.*, a therapy to compensate for defects in a gene) wherein genetically modified cells are immunoisolated "in a biocompatible membrane, thereby introducing a system that can provide ***sustained delivery*** of the desired gene product" (Tai *et al.*, abstract, emphasis added). Gene products include proteins, peptides, and enzymes which are usually not cytotoxic. To evaluate the model Tai *et al.* encapsulated fibroblasts which express human growth hormone. Tai *et al.* do not report that sustained delivery of human growth hormone by the encapsulated fibroblasts had a toxic effect on the encapsulated fibroblasts which expressed the hormone or any cytotoxic effect on the cells in the environment into which they were delivered. Indeed, human growth hormone is not a protein that exerts a cytotoxic effect.

Wei *et al.* describe fibroblasts transfected with a retroviral vector encoding Cytochrome P450. These fibroblasts were grafted into the brains of mice seeded with rat C6 gliomas, and subsequently, CPA was administered intrathecally or intratumorally (see page 973, right column, 1st paragraph, last sentence). Wei *et al.* states that "[i]t is not clear at this time whether [1] the tumor regression mediated by grafting of these cells into the tumor was caused by release of toxic CPA metabolites from P450 2B1-expressing fibroblasts and/or [2] by retrovirus-mediated transfer of the P450 2B1 gene to neighboring tumor cells" (Wei *et al.*, page 976, column 1).

If tumor regression was caused by release of toxic CPA metabolites from P450 2B1-expressing fibroblasts of Wei *et al.*, then intrathecally administered CPA is converted into cytotoxic metabolites. Cytotoxic metabolites produced under these circumstances can diffuse more easily and do not accumulate, or do so to a minor extent, in the presence of the fibroblasts. Thus, one of skill in the art would reasonably expect that the cytotoxic metabolites would exert less of a cytotoxic effect on non-encapsulated fibroblasts itself, than on encapsulated fibroblasts.

Alternatively, if tumor regression was caused by retrovirus-mediated transfer of the P450 2B1 gene to neighboring tumor cells in Wei *et al.*, then recombinant retroviruses which express the cytochrome P450 gene are released from the fibroblasts and infect tumor cells. After

infection, the retroviral genome including the cytochrome P450 gene is inserted into the genome of the tumor cells and subsequently expressed by the tumor cell. After expression of the gene in tumor cells, CPA is administered ***intratumorally*** and cytotoxic CPA metabolites are produced directly within the tumor and exert their cytotoxic effect directly in the tumor. In the case of intratumoral administration of CPA, cytotoxic metabolites are not released by the fibroblasts, and thus, cytotoxic effects are exerted minimally, if at all, on the fibroblasts.

Based on the combined teaching of Tai *et al.* and Merten *et al.* in view of Wei *et al.*, the skilled practitioner would have expected that cells which are stably transfected with the cytochrome P450 gene, and thus express Cytochrome P450 protein constitutively, and which are subsequently densely packaged into capsules, would immediately be killed by the higher concentration of cytotoxic metabolites produced by conversion of prodrug molecules within the capsules. In this case, no dilution effect takes place either by direct release of the toxic metabolites into the surrounding environment, as is the case with non-encapsulated fibroblasts or by release of recombinant retroviruses. In fact, the skilled practitioner would have assumed that conversion of prodrug into cytotoxic drug within capsules and by encapsulated cells, respectively, would have the same cytotoxic effect on the cells as exerted by the recombinant retrovirus-infected tumor cells intratumorally provided with prodrug of Wei *et al.*

For these reasons, the combined teaching of Tai *et al.*, Merten *et al.* and Wei *et al.* would not have motivated one of skill in the art to produce a capsule comprising a porous membrane formed by a polyelectrolyte complex which encapsulates cells which express cytochrome P450 "as a cell membrane-bound protein", wherein the porous membrane of the capsule is permeable to prodrug molecules and the cells are retained within the capsule. In addition, the combined teaching would not have provided a reasonable expectation that such encapsulated cells could be used to ablate tumor cells or treat tumors.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By Anne J. Collins

Anne J. Collins
Registration No. 40,564
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133
Dated: August 23, 2004